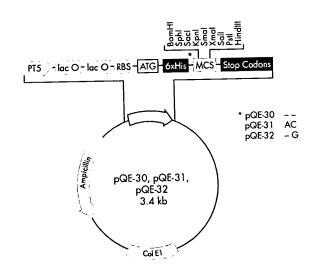
pQE-30, pQE-31, and pQE-32 Vectors

| Positions of elements in bases Vector size (bp) Start of numbering at Xhol (CTCGAG) T5 promoter/lac operator element T5 transcription start 6xHis-tag coding sequence Multiple cloning site Lambda to transcriptional termination region rrnB T1 transcriptional termination region ColE1 origin of replication | pQE-30 | pQE-31 | pQE-32 |
|--|-----------|-----------|-----------|
| | 3461 | 3463 | 3462 |
| | 1-6 | 1-6 | 1-6 |
| | 7-87 | 7-87 | 7-87 |
| | 61 | 61 | 61 |
| | 127-144 | 127-144 | 127-144 |
| | 145-192 | 147-194 | 146-193 |
| | 208-302 | 210-304 | 209-303 |
| | 1064-1162 | 1066-1164 | 1065-1163 |
| | 1638 | 1640 | 1639 |
| | 3256-2396 | 3258-2398 | 3257-2397 |
| β-lactamase coding sequence | 3256-2390 | 3230-2370 | 3237-2077 |



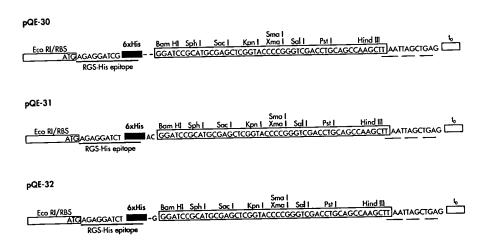
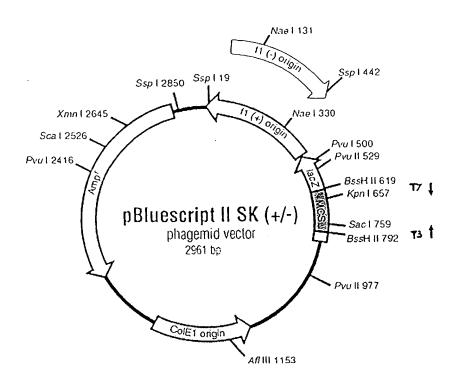
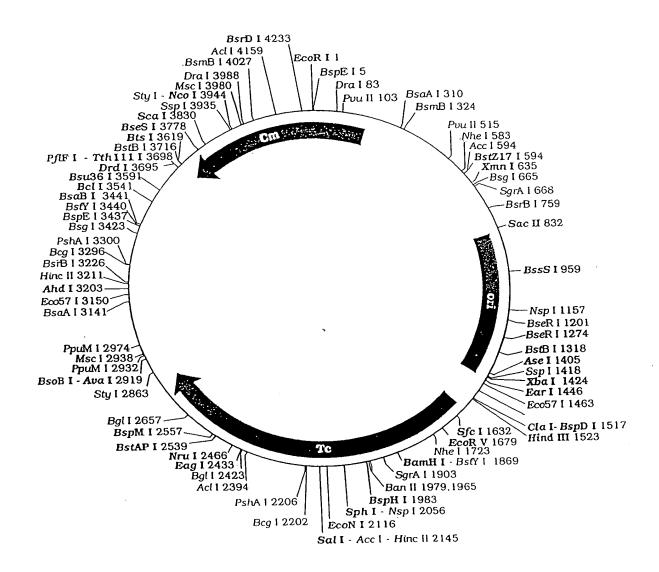


Figure 1



4,244 base pairs GenBank Accession / X06403

FIGURE 4



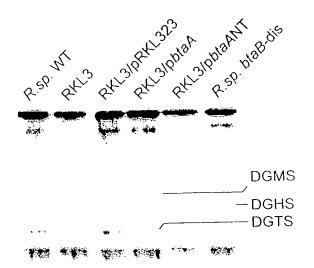
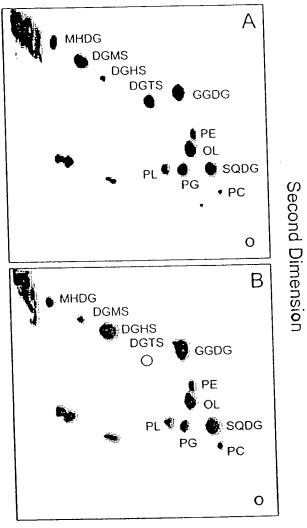


FIGURE 5



First Dimension

btaA gene cDNA Sequence

| • | 1-57 | gtgacge agttcgccct cacccacctg cccgccccgc cggttgcccg ccagatcggc |
|---|-----------|---|
| | 58-117 | geogeogtge accgeaegte getteteage geogaaggae tgatggageg gatgtteteg |
| | 118-177 | egeetettee aeggeetegt etateegeag atetgggagg ateeggeggt ggacatggeg |
| | 178-237 | gccctcgcca tccgcccgg ggaccggctg gtggccatcg cctcgggcgg ttgcaacgtg |
| | 238-297 | ettteetate teaegeaggg geegggeteg atectegeeg tggatetete geeegeecat |
| | 298-357 | gtggcgctgg ggcggctgaa getcgccgcc gcgcggacgc tgcccgacca tgccgccttc |
| | 358-417 | ttcgatctct tcggtcgcgc agacctgccc ggcaatgcgg ccctctacga ccgccacatc |
| | 418-477 | gcgcccgcgc tcgacggccg gagccgccgc tactgggagg cgcgcagccc cttcggccgg |
| | 478-537 | cgcatccage tgttcgageg eggettetae eggeaeggtg eceteggeeg etteategge |
| | 538-597 | geggeceata egetegegeg ggeegeggge acegacetge ggggetttet egactgtece |
| | 598-657 | gacategagg egeagegeag ettettetae geceatateg ggeegetett egaggegeee |
| | 658-717 | gtggtgcagg cgctcgcccg acggccggcc gcgctcttcg ggctggggat cccgccgcg |
| | 718-777 | caatatgege ttetggeggg agaeggegae ggegaegtge tgeeggtget gegeeagege |
| | 778-837 | ctccaccggc tgetctgtga ettccccctg cgcgagaact acttcgcctt ccaggccatc |
| | 838-897 | georgecget atecgeggee eggegaggge gegetgeege cetatetega acceaeegee |
| | 898-957 | ttcgagacgc tgcgcgagaa cgcgggccgg gtgcagatcg agaaccgcag cctgaccgag |
| | 958-1017 | gegetegegg eegaaceega ggagageate eaeggettea eeetgetega tgegeaggae |
| | 1018-1077 | tggatgacgg acgcgcagct gaccgcgctc tggcggcagg tgacgcgcac tgcagcgccg |
| | 1078-1137 | ggcgcgcggg tgatcttccg caccggcggg gcggccgacc tgctgcccggccgagtgccc |
| | 1138-1197 | gaggagatec tegggeactg gegegeegae egggeggegg gaeaggeggg ceatgeege |
| | 1198-1252 | gaccettegg egatetaegg eggetteeae etetaeegge ggagggaege eatga |

btaB gene cDNA Sequence

| 1-60 | atgaccgacg ccacccatgc ggcgctgatg gacgcgacct accgccacca gcgccggatc |
|-----------|---|
| 61-120 | tacgacgtca cgcggcggca cttcctgctc ggccgcgacc ggctgatcgc cgagctcgac |
| 121-180 | ccgcccccg gcgcccgggt gctcgagatc gcctgcggca cggggcgcaa cctcgacctg |
| 181-240 | ateggeegge getggeeegg etgeeggete teggggeteg acatetegea ggagatgetg |
| 240-300 | gcctcggccc gcgcgctct gggccggcgc gcgacgctgg cgctcggcga tgccacccgg |
| 301-360 | ttcgaggccc tgcccctctt cggcaccgac cggttcgagc ggatcgtcct ctcctacgcg |
| 361-420 | ctctcgatga tccccgactg gcgcgaggcc ctgcgtgagg cggcgcttca tctcgtgccg |
| 421-480 | ggggggcggc tgcatgtcgt cgacttcggc gatcaggcgg gcctgcccgg ctgggcccgc |
| 481-540 | geoggeetge geggetggat egggegette eacgteaege egegegaega tetgggeaeg |
| 541-600 | geaetgggeg aaacggeget egggateggg ggetatgeeg aataceggte eeteggegg |
| 601-660 | ggatatgcga ttctcggcac gctcacgcgg tgagagatcc cctgccctgc |
| 661-720 | tgtctgcccg caggcgaccg gccgcgcgac ggccggcctg cgggcgatcc ggcgcactga |
| 721-780 | aggeceggeg egtegegeg ggggaegtag eeegeagegg eaageggeeg acagageetg |
| 781-840 | acagaccett cacegetece getecegate gegtetegag cegetettec agageteage |
| 841-900 | cctcgaggga aagccctctg gcccgacggg caaattgtcc gggatctcta atcgggaaat |
| 901-960 | tggtcggage gagaggatte gaaceteega eeeeetgete eegaageagg tgegetacea |
| 961-1020 | ggetgegeta egeteegace ttggegtgeg gattataggg tegegeatee gaatgeaagg |
| 1021-1080 | gggtccgaac gcaattcgct acggagtgtc tcgcgtctcg cggcggcgca gaaggcgcgg |
| 1081-1140 | catgaggece acetegggee geaggeget etggetegee gggeggttet eegacaegtt |
| 1141-1200 | geggegegat tegeggeega egatatagag geegetegeg atgatgaeee eegeeegae |
| 1201-1255 | ccaggtccag acgtcggacc gctcgccgaa gatgagccag ccgaagatcc ctgac |

btaA gene Amino Acid Sequence

1-50 MTQFALTHLP APPVARQIGA AVHRTSLLSA EGLMERMFSR LFHGLVYPQI
51-100 WEDPAVDMAA LAIRPGDRLV AIASGGCNVL SYLTQGPGSI LAVDLSPAHVAL
101-150 GRLKLAAART LPDHAAFFDL FGRADLPGNA ALYDRHIAPA LDGRSRRYWE
151-200 ARSPFGRRIQ LFERGFYRHG ALGRFIGAAH TLARAAGTDL RGFLDCPDIE
201-250 AQRSFFYAHI GPLFEAPVVQ ALARRPAALF GLGIPPAQYA LLAGDGDGDV
251-300 LPVLRQRLHR LLCDFPLREN YFAFQAIARR YPRPGEGALP PYLEPTAFET
301-350 LRENAGRVQI ENRSLTEALAA EPEESIHGFT LLDAQDWMTD AQLTALWRQV
351-400 TRTAAPGARV IFRTGGAADL LPGRVPEEIL GHWRADRAAG QAGHAADRSA

btaB gene Amino Acid Sequence

1-50 MTDATHAALM DATYRHQRRI YDVTRRHFLL GRDRLIAELD PPPGARVLEI
51-100 ACGTGRNLDL IGRRWPGCRL SGLDISQEML ASARARLGRR ATLALGDATR
101-150 FEALPLFGTD RFERIVLSYA LSMIPDWREA LREAALHLVP GGRLHVVDFG
151-200 DQAGLPGWAR AGLRGWIGRF HVTPRDDLGT ALGETALGIG GYAEYRSLGG
201-210 GYAILGTLTR

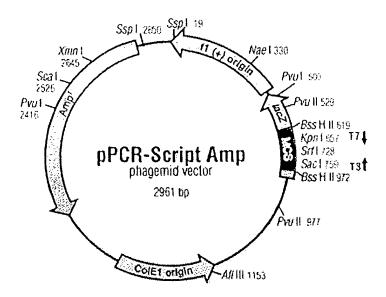
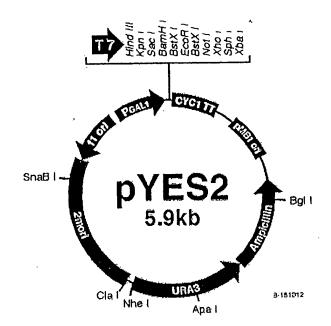


FIGURE 12

Comments for pYFS2: 5867 nucleotides

FIGURE 13

GAL I promoter: bases 1-452 T7 promoter/primitry site: bases 476-495 Multiple cloning site: bases 502-601 CYCI transoription terminator: bases 608-857 pMB1 (pLIC-derived) origin: bases 1039-1712 Ampicilia resistance gene: bases 1857-2717 UFA2 gene: bases 2735-3842 2 micron origin: bases 3840-5317 11 origin: bases 5985-5840



Mutagenesis Oligonucleotide btaA-L9I

5'-CGC CCT CAC CCA C<u>AT T</u>CC CGC CCC GC-3'

and its reverse complement:

5'-GCG GGG CGG G \underline{AA} \underline{T} GT GGG TGA GGG CG-3'

Mutagenesis Oligonucleotide btaA-A201G

5'-GAC TGT CCC GAG ATC GAG GGC CAG CGC CAG C-3'

and its reverse complement:

5'-GCT GGC GCT G<u>GC C</u>CT CGA TCT CCG GAC AGT C-3'

Mutagenesis Oligonucleotide btaA-S399T

5'-GCC GCC GAC CGT <u>ACG</u> GCG ATC TAC GG-3'

and its reverse complement:

5'-CCG TAG ATC GC<u>C GT</u>A CGG TCG GCG GC-3'

Mutagenesis Oligonucleotide btaB-T13S

5'-GCT GAT GGA CGC G<u>TC C</u>TA CCG CCA CCA G-3'

and its reverse complement:

5'-CTG GTG GCG GTA GGA CGC GTC CAT CAG C-3'

Mutagenesis Oligonucleotide btaB-I115L

5'-CGG TTC GAG CGG <u>CTC</u> GTC CTC TCC TAC GC-3'

and its reverse complement:

5'-GCG TAG GAG AGG AC<u>G AG</u>C CGC TCG AAC CG-3'

Mutagenesis Oligonucleotide btaB-G206A

5'-GGA TAT GCG ATT CTC GCC ACG CTC ACG CG-3'

and its reverse complement:

5'-CGC GTG AGC GT<u>G GC</u>G AGA ATC GCA TAT CC-3'

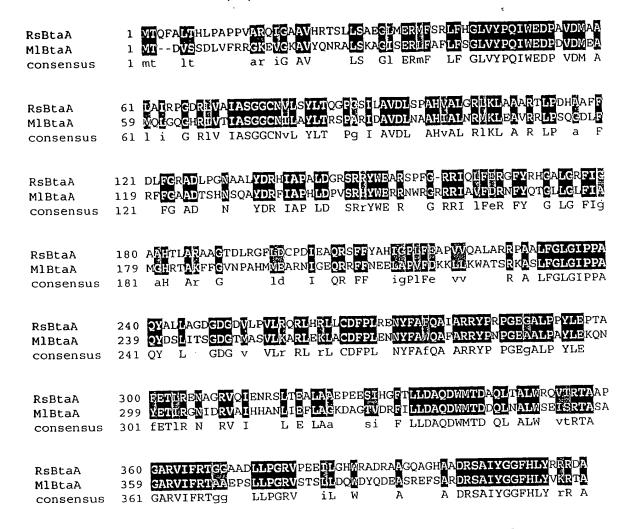


FIGURE 20

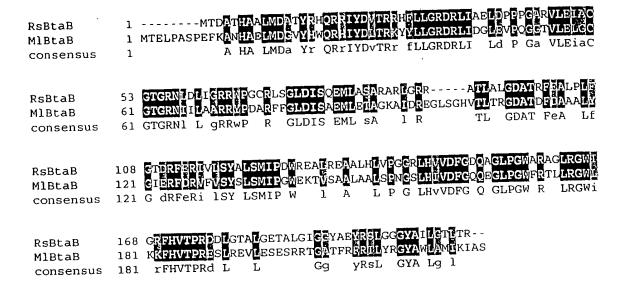


FIGURE 21

Ml-btaA gene sequence

| 269421 | atgacggacgtctcctcggatctggtttttcgccgcggcaa |
|--------|--|
| 269461 | ggaagttggaaaggccgtctaccagaaccgcgcgctttccaaagccggcatctccgagcg |
| 269521 | gctgttcgccttcctgttttccggcctcgtctatccgcagatctgggaagaccccgatgt |
| 269581 | cgacatggaggccatgcagcttggtcagggccatcgcatcgtcacaatcgcttccggcgg |
| 269641 | ctgcaacatcctcgcctacctcacccgttcgccggcacggatcgacgccgtcgacctcaa |
| 269701 | cgccgccacatcgcgctgaaccgcatgaagctggaggcggtgcgccgtctgccctcgca |
| 269761 | gggcgatctgttccgctttttcggcgccgccgacaccagccacaattcgcaagcctatga |
| 269821 | ccgctttattgcgccgcatctcgatccggtcagccgccactattgggagcgccgcaactg |
| 269881 | gcgtggtcgccggcgcatcgccgtcttcgaccgcaatttctaccagaccggcctgctcgg |
| 269941 | cetgttcategecatgggccategcacggcgaaattetteggegtcaaceeggcccacat |
| 270001 | gatggaagccaggaatatcggcgagcagcgccgcttcttcaacgaggagctggcgccggt |
| 270061 | cttcgacaagaagcttttgaaatgggcgacctcgcgtaaggcctcgctgttcggcctcgg |
| 270121 | cattccgccggcgcagtacgattccctgatcacctcaggcgacggcaccatggccagcgt |
| 270181 | tctgaaggcccggctggaaaagctcgcctgcgattttcccctggaaaacaattatttcgc |
| 270241 | ctggcaggcttttgcccgccgctatccaaatcccggtgaggccgccctgcccgcctatct |
| 270301 | ggaaaagcagaactacgaaaccatccgcggcaatatcgaccgcgtcgccatccaccatgc |
| 270361 | caatctgatcgaattcctcgccggcaaggacgcgggcaccgtcgatcgcttcatcctgct |
| 270421 | cgatgcgcaggactggatgaccgatgaccagctcaacgcgctgtggtcggaaatcagccg |
| 270481 | cacegceteegeaggegeeggteatetteegeacegeegageeeageetgetgee |
| 270541 | aggccgcgtctcgacctcgctgctcgaccagtgggactatcaggacgaggcgtcgcgcga |
| 270601 | atteteggeaeggaeegtteggeeatetatggeggetteeaeetetatgtgaagegeae |
| 270661 | ggca <u>tga</u> |
| | |

FIGURE 22

Ml-btaB gene sequence

| atgaccgagctgccggccagccccgaattcaaggccaatcatgccgaactg |
|--|
| atggacggcgtctaccactggcagcgccacatctatgacctgactcgcaaatactatctg |
| ctcggccgcaccggctgatcgatgggcttgaggtgccgcaaggcggcaccgtgctggaa |
| ctcggctgcggcaccggccgcaacatcatcctggccgccgccgctaccctgatgcccgc |
| ttcttcggcctggatatctcggccgagatgctggagacggccggc |
| gaaggcctgtccggccacgtaacgctgacacgaggcgacgccaccgatttcgacgccgcg |
| gcactttacggcatcgagcgcttcgaccgcgtcttcgtctcctattcgctgtcgatgatc |
| ccaggctgggaaaagacggtgtcggcggcactcgccgcactatcccccaacggctcgctg |
| cacategtegattteggeeageaggaaggeetacegggetggtteegtacettgetgege |
| ggttggctgaaaaaattccacgtaacgccgcgtgaatcgctgcgcgaagttctggaatcg |
| gaatctcggcgaaccggcgcaaccttccgtttccgcacgctttatcgcggttacgcctgg |
| ctggcgatgatcaagatcgccagc <u>taa</u> |
| |

FIGURE 23

Agrobacterium tumefaciens BtaA DNA

| 1201 | 1141 | 1081 | 1021 | 1967 | £06 | 841 | 781 | 721 | 661 | 601 | 541 | 481 | 421 | 361 | 301 | 241 | 181 | 121 | 61 | 1 |
|------|------|------|------|-------------|-----|----------|-------------------|-----|--------|-----|-------------|-------------|-----|--------------|--------------|-----|-------------|-----------------------|----------|-----|
| cgc | gac | gca | atg | H | gaa | cgc | gaa | gac | cgc | gac | Cac | tcg | ctg | ttc | aac | 010 | ctt | tcc | ctc | atg |
| tcg | atc | cgc | acg | tcc | aag | cgt | aag | gag | tgg | gaa | atc | gtg | gat | 9 9 C | aag | tcg | 9 9c | 99 € | cag | acg |
| gcc | cgc | gtc | gat | cgc | atc | tat | Ctt | ctg | ctg | Cag | atg | ttc | gcc | cgc | ctg | Cgc | gaa | CtC | Cac | agt |
| att | aac | atc | gtg | aag | cgc | CCC | gcc | gca | acg | cgc | gcc | gac | acg | gcc | aag | aac | 9gc | gtc | aag | 909 |
| tat | cag | ttc | cag | 609 | aac | gag | tgc | agc | aag | cag | cgc | agg | acc | 99 C | ctc | CEg | Cac | tat | gca | gca |
| | | | | | | CCG | | | | | | | | | | | | | | |
| | | | | | | cat | | | | | | | | | | | | | | |
| ttc | tat | gcg | gag | 99 C | 909 | gag | CCG | agc | agc | gac | 9 90 | tac | tac | agc | ttc | atc | gtc | atc | aaa | acc |
| H | 7 | S | 딥 | П | 7 | ggt | 7 | 7 | G | Ö | g | 99 | 99 | D C | O | at | 2 | gg | <u> </u> | gc |
| 2 | PE | E | 90 | 2 | Н | 326 | 9 | S | H | 9 | a | C | ධු | 9 | 2 | ţ | 40 | 5 | 26 | tc |
| | | | | | | ctg | | | | | | | | | | | | | | |
| | | | | | | CCC | | | | | | | | | | | | | | |
| | | | | | | gct | | | | | | | | | | | | | | |
| 4.4 | • • | ., | • • | _ | | tat | 1 3 | 1) | " | П | a, | П | П | П | П | n | n | \mathbf{G}^{\prime} | KC) | Ω |
| 9 | ä | Š | ä | 2 | S | | 2 | Ц | H | 5 | 2 | gt | 2 | gt | g | G | 00 | 26 | # | aa |
| | • • | • • | 17 | TT. | 11 | <u>u</u> | u | () | L | | u. | n | (1 | n | ΓŤ | n | U. | C | ח | 0 |
| ā | 7 | ğ | H | Ö | # | | Ö | Ö | | Ô | S | S. | d | ሽ | ű | 0 | G | מ | 0, | D |
| | H | Ħ | מ | g | R | gaa | \mathcal{C}_{i} | Ö | ה ה | 6 | 99 | 90 | gt | 2 | G | 7 | 0,0 | ß | t | 2 |
| | | | | | | tat | | | | | | | | | | | | | | |
| • | gac | 5 | ggg | tgg | Ctg | tac | gcg | Ctg | tat | gtg | ctg | 9 90 | att | cat | cac | Ctd | tat | gag | ttt | tta |

Agrobacterium tumefaciens BtaA protein

MTSAAPKTGFSKNTKLKSALLQHKALSKSGLSERFFGVLFSGLVYPQIWEDPEIDMEA MELGEGHRIVTIGSGGCNMLAYLSRNPASIDVVDLNPHHIALNKLKLAAFRHLPAHQD VVRHFGRAGTRSNSVGYDRFIAEHLDATTKAYWSKRTLSGRRRISVFDRNIYRTGLLG RFIGAGHIMARLHGVKLTEMAKTRTLDEQRQFFDSKVAPLFDKPVVRWLTKRKSSLFG LGIPPRQYDELASLSSDGTVASVLKERLEKLACNFPLSDNYFAWQAFARRYPEPHEGA LPAYLKPEYYEKIRNNTARVAVHHATYTELLSRKPANGVDRYILLDAQDWMTDVQLNE LWSQISRTAASGARVIFRTAAEKSVIEGRLSPDIRNQWVYLEERSNELNAMDRSAIYG GFHIYQRAMA

Agrobacterium tumefaciens BtaB DNA

```
atgaaaacca tcggcgagaa tgtcggcctt gcagacagcg cgcatgcggg cttgatggac cgcatgtatc gccaccagcg ccatatctac gatatcaccc gcaaatatta tcttctgggc 121 cgtgaccgga ccatttccgg cctcgacgtg ccaaagggcg gcacgctgct ggaaatcggc 121 tgcggcaccg gccgcaacct gctgctggcc agccgccggt ttcccgacgc caaactcttc 241 ggcctcgata tatcagccga aatgctgctg accgcctccg agaattttgc cggcaaagcg 301 gagcgaccca ttctgcgtgt cgccgatgcc accgcttcc ggtcttcgga attcggccag 361 cccgatggct tcgaccgcgt catgatcct tatgcgctgt cgatgatacc ggactgggaa 421 aaagcgatcg aacaggcgt cgcgatgcc accgctttcc ggttcgctgca tatcggcaag 421 ttcggccagc aggaacagtt gccgaagtgg ttccgcacgc ttcttcaagc ctggctaccc 121 cgcttcacg ttaccgcccg cgcaaatctc cgttacgttc tcgccaatat ggccgacgt cgcgaagtgg ttccgcacgc ttcttcaagc ctggctcacc 121 cgcttcacg ttaccgcccg cgcaaatctc cgttacgttc tcgccaatat ggccggccgt cgaaggggaa accgctggaaccgg ttgccgaagccggaaacc gcgaaggggaa acgcatggcg ggctgtcatc acgcttccgg ttgccgaagc cccgcagccg aagatccacc gcttattggc tgacgcctga
```

Agrobacterium tumefaciens BtaB protein

MTDATHAALMDATYRHQRRIYDVTRRHFLLGRDRLIAELDPPPG ARVLEIACGTGRNLDLIGRRWPGCRLSGLDISQEMLASARARLG RRATLALGDATRFEALPLFGTDRFERIVLSYALSMIPDWREALR EAALHLVPGGRLHVVDFGDQAGLPGWARAGLRGWIGRFHVTPRD DLGTALGETALGIGGYAEYRSLGGGYAILGTLTR

Sinorhizobium meliloti BtaA DNA

octo atto acco acco cood ctg aac cat cat ttc gac ctg ccg ccg ccg agc cag agc cttc tga agc cttc aaa ctgac ccc ccc cgc cct cct ccc aggac aggac ccg ggc ggc gac ctc ctc ctc ttat ctc ctc ctc ctc acc a a constant of the constant o oggc oggc atta tatt tatt cogg ccc gag ccc gag ttat ttcaa 121 121 181 181 301 4421 601 721 721 721 721 721 721 721 721

Sinorhizobium meliloti BtaA protein

MTDFAPDAGFGKKNPKLKSALLQHKALSPAGLSERLFGLLFSGLVYPQIWEDPIVDME AMQIRPGHRIVTIGSGGCNMLTYLSAEPARIDVVDLNPHHIALNRLKLSAFRHLPSHK DVVRFLAVEGTRTNGQAYDVFLAPKLDPATRAYWNGRDLTGRRRIGVFGRNVYRTGLL GRFISASHALARLHGINPEDFVKARSMREQRQFFDDKLAPLFERPVIRWITSRKSSLF GLGIPPQQFDELASLSREKSVAAVLRNRLEKLTCHFPLRDNYFAWQAFARRYPRPDEG ELPPYLQASRYEAIRDNAERVEVHHASFTELLAGKPAASVDRYVLLDAQDWMTDQQLN DLWTEITRTADAGAVVIFRTAAEASILPGRLSTTLLDQWYYDAETSMRLGAEDRSAIY GGFHIYRKKA

Sinorhizobium meliloti BtaB DNA

```
atgagcgccg tgcagaccgc gaatgaaagc cacgctcatc tgatggaccg catgtatcgc 61 taccagcggt acatctatga tttcactcgc aaatactatc tcttcggccg tgacacgctg 121 atccgtgaac tgaacccgcc gccaggcgca tcggtgctgg aagtcggctg cggcacgggc 181 cgcaatctcg ccgtgatcgg ggatctctac cccggtgcgc gcctcttcgg cctcgatatc 182 tcggccgaaa tgctggcgac cgccaaagcc aagctccggc gccaaaatcg gccggacgca 183 gtgttgcggg tcgccgacgc gacgaatttc accgccgct cattcgatca ggaaggcttc 183 gaccggatcg tcatttccta cgcctttcc atggttcccg aatgggaaaa ggcggtcgat 184 gaaggttggc cggccgct ccgcggcgc tcgctgcata tcgccgact cggccagcag 184 gaaggttggc cggccggct ccgccgcttc ctccaggcct ggctcagacg cttccacgtc 184 acgccgcgcg aaacgctttt cgatgtgatg cgcaaaagag ccgagagaaa cggagcggcg 184 acgccgcgcg aaacgctttt cgatgtgatg cgcaaaagag ccgagagaaa cggagcggcg 185 acgccggca gatcgctgag acgaggttat gcctggcttg tcgtctatcg ccgcgcgca 185 acgccgcgca gatcgctgag acgaggttat gcctggcttg tcgtctatcg ccgcgcgca 185 acgccgcgca 185 acgccgca 185 acgccgcgca 185 acgccgcgca 185 acgccgcgca 185 acgccgcgca 185 acgccgca 185 acgcc
```

Sinorhizobium meliloti BtaB protein

MSAVQTANESHAHLMDRMYRYQRYIYDFTRKYYLFGRDTLIREL NPPPGASVLEVGCGTGRNLAVIGDLYPGARLFGLDISAEMLATA KAKLRRQNRPDAVLRVADATNFTAASFDQEGFDRIVISYALSMV PEWEKAVDAAIAALKPGGSLHIADFGQQEGWPAGFRRFLQAWLR RFHVTPRETLFDVMRKRAERNGAALEVRSLRRGYAWLVVYRRAA P

Figure 32

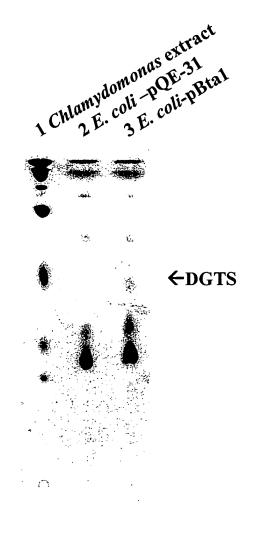


Figure 33

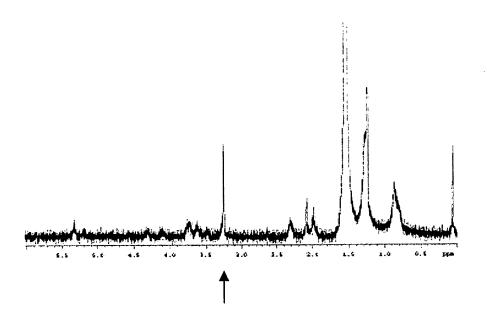
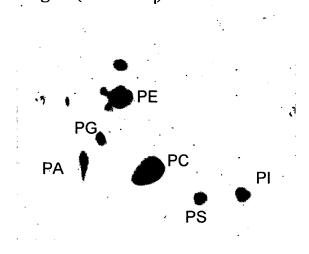


Figure 34

A. Vogels (20 mM P)



B. Vogels -P +MES (0.01 mM P_i)

